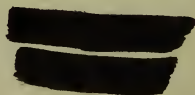


THE UNIVERSITY
OF ILLINOIS
LIBRARY

630
Un3an
no.132-42





NOTICE: Return or renew all Library Materials! The Minimum Fee for each Lost Book is \$50.00.

The person charging this material is responsible for its return to the library from which it was withdrawn on or before the **Latest Date** stamped below.

Theft, mutilation, and underlining of books are reasons for disciplinary action and may result in dismissal from the University.

To renew call Telephone Center, 333-8400

UNIVERSITY OF ILLINOIS LIBRARY AT URBANA-CHAMPAIGN

NOV 14 1990

AUG 24 1990

FEB 07 1996

MAR 01 2001

MAY 08 1996

APR 19 1996

APR 20 2001

MAY 11 1996

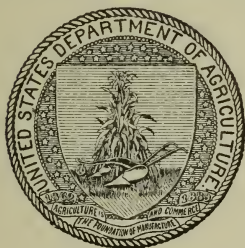
Issued April 1, 1911.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY.—BULLETIN 133.
A. D. MELVIN, CHIEF OF BUREAU.

THE DETERMINATION OF NICOTIN
IN NICOTIN SOLUTIONS AND
TOBACCO EXTRACTS

BY

ROBERT M. CHAPIN,
Senior Biochemist, Biochemic Division.



WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1911.

THE BUREAU OF ANIMAL INDUSTRY.

Chief: A. D. MELVIN.

Assistant Chief: A. M. FARRINGTON.

Chief Clerk: CHARLES C. CARROLL.

Animal Husbandry Division: GEORGE M. ROMMEL, chief.

Biochemic Division: M. DORSET, chief.

Dairy Division: B. H. RAWL, chief.

Inspection Division: RICE P. STEDDOM, chief; MORRIS WOODEN, R. A. RAMSAY,
and ALBERT E. BEHNKE, associate chiefs.

Pathological Division: JOHN R. MOHLER, chief.

Quarantine Division: RICHARD W. HICKMAN, chief.

Zoological Division: B. H. RANSOM, chief.

Experiment Division: E. C. SCHROEDER, superintendent.

Editor: JAMES M. PICKENS.

LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY,
Washington, D. C., January 11, 1911.

SIR: I have the honor to transmit herewith a paper on "The Determination of Nicotin in Nicotin Solutions and Tobacco Extracts" by Mr. Robert M. Chapin, Senior Biochemist in the Biochemic Division of this bureau, and to recommend its publication in the bulletin series of the bureau.

This paper describes an accurate and rapid method for the determination of nicotin, the method being applicable especially to the examination of nicotin solutions and tobacco extracts which are used in the official dipping of sheep and cattle for scabies. The method presented by Mr. Chapin has many advantages over the Kissling method which is now generally employed, and it is believed that the paper will be of interest and value not only to Federal and State officers who have to deal with nicotin solutions, but also to manufacturers who are endeavoring to produce standard and reliable articles.

Respectfully,

A. D. MELVIN,
Chief of Bureau.

HON. JAMES WILSON,
Secretary of Agriculture.

CONTENTS.

	Page.
Introductory.....	5
The Kissling method.....	6
The petroleum ether method.....	8
The silicotungstate method.....	10
Development of the quantitative method.....	12
Analytical results obtained by the silicotungstate method.....	14
The determination of nicotin in commercial preparations.....	15
The effect of extraneous bases.....	16
Tests on a rapid modification of the method.....	17
The steam distillation of nicotin preparations.....	20
Summary and codified directions for routine work.....	21

THE DETERMINATION OF NICOTIN IN NICOTIN SOLUTIONS AND TOBACCO EXTRACTS.

INTRODUCTORY.

Nicotin has long been regarded as a valuable agent for the extermination of certain species of insects infesting plants and animals. For several years proprietary nicotin solutions and tobacco extracts have been permitted by the Bureau of Animal Industry¹ for use in the official dipping of cattle and sheep affected with or exposed to scabies. Nicotin baths employed in official dipping are required to possess a minimum strength of either 0.05 or 0.07 of 1 per cent of nicotin, according to circumstances. Upon the Biochemic Division of the Bureau of Animal Industry has accordingly devolved the task of examining a considerable number of such preparations, including both samples from manufacturers who have desired to obtain permission for the use of their products in official dipping, and samples of dips actually put to such use, transmitted by bureau inspectors in the field.

The method hitherto employed by the bureau for the determination of nicotin is the Kissling method, adopted several years ago by the Association of Official Agricultural Chemists as the standard method for nicotin in tobacco and tobacco products. The Kissling method in experienced hands and in the absence of certain interfering substances unquestionably yields accurate results. But it contains a number of pitfalls for the unwary; it is cumbersome, tedious, and expensive. The bureau is now in possession of a method which appears to yield results of a higher degree of accuracy than the classical Kissling method, at a less expenditure of time, chemicals, and expensive apparatus, and to yield such results in the hands of any competent chemist of ordinary training. Therefore this paper is offered in order that the method may be available to manufacturers, who are naturally interested in maintaining their preparations for use in official dipping at the standard required by the bureau, as well as to the considerable number of manufacturing, State, and Government chemists who are concerned with the examination

¹ Bureau Animal Industry Order 143, Regulations 25 and 33, and Amendment 1.

of nicotin preparations used as general insecticides upon both animals and plants.¹

THE KISSLING METHOD.

The Kissling method as adopted by the Association of Official Agricultural Chemists is carried out as follows:²

Weigh from 5 to 6 grams of tobacco extract, or 20 grams of finely powdered tobacco, which has been previously dried at 60° C., so as to allow it to be powdered, into a small beaker. Add 10 c. c. of the alcohol-soda solution [6 grams NaOH, 40 c. c. water, 60 c. c. of 90 per cent alcohol] and follow, in the case of the tobacco extract, with enough chemically pure powdered calcium carbonate to form a moist but not lumpy mass. Mix the whole thoroughly. Transfer this to a Soxhlet extractor and exhaust for about five hours with ether. Evaporate the ether at a low temperature by holding over the steam bath, and take up the residue with 50 c. c. of the dilute sodium hydroxid solution [4 grams NaOH, 1000 c. c. water]. Transfer this residue by means of water to a Kjeldahl flask, capable of holding about 500 c. c., and distil in a current of steam, using a condenser through which water is flowing rapidly. Use a three-bend outflow tube, a few pieces of pumice, and a small piece of paraffin, to prevent bumping and frothing. Continue the distillation until all the nicotin has passed over, the distillate usually varying from 400 to 500 c. c. When the distillation is complete, only about 15 c. c. of the liquid should remain in the distillation flask. Titrate the distillate with standard sulphuric acid, using phenacetolin or cochineal as indicator. One molecule of sulphuric acid is equivalent to two molecules of nicotin.

The Kissling method involves two series of operations, (1) extraction with ether, (2) distillation with steam and titration. For convenience of discussion these two series of operations will be considered in reverse order.

Titration and steam distillation.—None of the indicators tried in this laboratory have given perfect satisfaction in the titration of distillates from commercial nicotin preparations, although some show a sharp enough change of color when used with solutions of pure nicotin. In practice, as noted by other investigators, the end point is apt to be rather indefinite, requiring some care and experience on the part of the operator if accurate results are to be obtained. A freshly prepared alcoholic solution of phenacetolin has yielded the best results in this laboratory, the practice here having been to distil over the nicotin into a measured amount of standard sulphuric acid, and to titrate back the excess of acid.

The chief drawback to the operation, however, is the fact that it reckons as nicotin all alkaline substances which may gain access to

¹ In the issue of the *Chemiker-Zeitung* of January 10, 1911 (Jahrgang XXXV, nummer 4, seite 30), in an article entitled "Zur Bestimmung des Nicotins in konzentrierten Tabaksäften," Dr. Johannes Schröder reviews the most recent work published upon the determination of nicotin, notes some inadequacies in methods at present in vogue and forcibly presents the pressing need for a method which, being accurate, practical, and universally employed, shall constitute a standard method for the valuation of a class of preparations which, according to figures cited, are employed in large quantities.

² U. S. Department of Agriculture, Bureau of Chemistry Bulletin 107, Revised 1910.

the receiver during steam distillation: Alkali extracted from the glass condenser, if such is used; alkali entrained by the current of steam; ammonia, amines, and pyridin bases, if such were present in the original extract and not removed during preceding operations; ammonia from the decomposition of proteid matter of the extract which may have reached the distillation flask; all are possible sources of error which may cause too high results to be obtained. Disregarding minor sources of error, it is certain that all ammonia, amines, and pyridin bases must be eliminated before the distillation with steam is undertaken. This end is not accomplished by the evaporation at a low temperature over the steam bath directed for the removal of ether, for experiments in this and other laboratories have shown that such evaporation must cease while there is still a considerable quantity of ether remaining over the alcoholic-aqueous residue in the extraction flask or loss of nicotin will occur.

Extraction with ether.—From the preceding it is clear that if volatile alkalis other than nicotin are to be eliminated at all during the Kissling process it must be during the operation of extraction with ether. That such is actually the case may be plainly shown by a slip of litmus paper inserted in the top of the condenser during extraction. The official directions of the Association of Official Agricultural Chemists prescribe an extraction time of five hours, which period is undoubtedly sufficient to extract all the nicotin. It is not, however, in many cases nearly long enough to volatilize all ammonia and amines, particularly if the condenser used is of the spiral form from which evolved vapors can not find a ready escape. The process of extraction must be continued until a strip of red litmus paper inserted in the upper end of the condenser retains its color permanently. The length of time required for such a complete elimination of extraneous volatile bases is naturally very variable, but an extraction period of more than 15 hours has sometimes proved necessary in this laboratory. It is to be noted that in this way only ammonia and amines are eliminated. Pyridin bases, on account of their high boiling points and great solubility in ether, remain behind with the nicotin.

The Kissling method, therefore, may yield accurate results, but it yields them only in the absence of certain interfering substances and only in the hands of a chemist of special experience. It is, moreover, highly probable that in the case of commercial nicotin preparations the apparently accurate results obtained are in reality simply a nice balancing of errors. In the necessary manipulation and during the long period of extraction often required, a considerable amount of ether is lost. Bertrand and Javilier state that their experiments show that in the distillation of ethereal solutions of nicotin, between 1 and 2 milligrams of the alkaloid accompany each 100 c. c. of

ether distilled, the loss becoming still more notable in the case of fairly concentrated solutions. In a properly executed determination by the Kissling method the inevitable loss of small amounts of nicotin is probably fairly well balanced by the equally inevitable retention of small amounts of ammonia and amines.

Aside from the question of accuracy, other factors, such as the amount of personal attention required, the employment of expensive apparatus, and the considerable loss of ether which inevitably occurs, contribute to make the process an expensive one, particularly when in view of the rather loose agreement between check determinations four or five repetitions may be necessary in order to obtain a thoroughly substantiated result.

THE PETROLEUM ETHER METHOD.

About 10 years ago Toth¹ employed a method for the determination of nicotin in tobacco, which appeared to give good results. The tobacco leaves were rubbed up in a porcelain mortar with a small quantity of strong caustic-soda solution, then thoroughly incorporated with plaster of Paris until a dry powder resulted. The latter was brought into a stoppered glass cylinder and thoroughly shaken with a definite volume of a mixture of petroleum ether and ethyl ether. After subsidence an aliquot of the ether was pipetted out, treated with indicator and excess of standard acid, and finally titrated back with standard alkali. Toth found that when ammonium salts were present only a very little ammonia was dissolved by the ether, and that the trace so dissolved could be readily removed by blowing air through the ether for a short time, though with some danger of simultaneous loss of nicotin.

In searching for an improvement on the Kissling method first attempts were directed toward a modification of Toth's method along the following lines:

To a weighed amount of nicotin solution or tobacco extract was added about 4 c. c. of kerosene, followed by a few grams of calcium hydrate or a very few cubic centimeters of a nearly saturated caustic soda solution. After thorough mixing, plaster of Paris, previously moistened with kerosene, was added in liberal excess of the quantity needed to combine with all water present, and the mass was mixed to uniform consistency. During the process of dehydration the kerosene previously added would presumably act as a solvent for liberated nicotin and so prevent loss by volatilization. The mixture was then transferred to the capsule of a Soxhlet apparatus and extracted with petroleum ether. On account of the total absence of

¹ Toth, Julius. Neue Methode zur Bestimmung des Nicotins im Tabak und in den wässrigen Auszügen der Tabakblätter. *Chemiker-Zeitung*, Jahrgang 25, No. 57, p. 610, July 17, 1901.

water and the slight degree of solubility of ammonia and amines in petroleum ether, these substances were rapidly evolved from the top of the condenser, and a slip of red litmus paper placed therein retained its color after a very much shorter period of extraction than is required in the Kissling method.

The petroleum ether extract remaining in the extraction flask was transferred to a separatory funnel and shaken out with a measured quantity of standard acid, followed by two or three washings of water. The excess of acid in the whole aqueous extract was lastly titrated back with standard alkali, following the method formerly used by Loesch.

The following table will illustrate the character of the results obtainable by this method:

TABLE 1.—*Comparative results of Kissling and petroleum ether methods on commercial nicotin solutions and tobacco extracts.*

Character of material.	Kissling method.	Petroleum ether method.
Tobacco extract, No. 754.....	2.92 2.88	2.98 2.92 2.96
Nicotin solution, No. 755.....	39.80 40.03 40.75	39.80 40.45
Nicotin solution, No. 810.....	38.89 39.54 39.78 39.42	40.51 40.08 40.42
Tobacco extract, No. 834.....	2.91 2.84	2.98 2.88 3.03
Nicotin solution, No. 835.....	41.40 40.84 40.62 40.29	40.70 40.91
Nicotin solution, No. 841.....	40.29 39.86	40.49

The method appears, therefore, to yield results which check very well with those obtained by the Kissling method. Its positive advantages are (1) much saving of time and expense by the replacement of a long period of extraction with ethyl ether by a short period with petroleum ether, and (2) elimination of the operation of steam distillation. But the proper preparation of the sample for extraction requires considerable manipulation, and the method is still a volumetric one, involving the use of a somewhat indefinite end point. Moreover, the presence of pyridin bases will lead to false results exactly as in the case of the Kissling method, and in addition a new source of error resulting from the possible presence of certain nonvolatile alkaloids is introduced. Hence the method described has been but little used in practice.

Incidentally Table 1 may serve to illustrate the rather wide variation between check determinations by the Kissling method when

applied to commercial preparations. A difference in duplicate results of 1 per cent of the amount of nicotin operated upon must be expected to occur regularly in routine work, while a difference of 2 per cent is not unusual.

THE SILICOTUNGSTATE METHOD.

In 1909 Bertrand and Javillier¹ published a paper on the use of silicotungstic acid as an aid in the quantitative determination of nicotin, employing it in the examination of tobacco. The first steps in their method were to extract nicotin by boiling the tobacco leaves with dilute hydrochloric acid, then to precipitate the nicotin by the addition of silicotungstic acid. The precipitate of nicotin silicotungstate, after washing, was brought into a flask, and the nicotin, liberated by an addition of calcined magnesia, was distilled over with steam, and determined in the distillate by titration with standard acid.

The method resembles the Kissling method to the extent that the nicotin is finally determined by titration after distillation with steam, and accordingly it is subject to several similar possible inaccuracies. It successfully eliminates the effect of ammonia present as such or as ammonium salts, for ammonium silicotungstate is soluble in water. But silicotungstic acid, like some other reagents for alkaloids, is also a precipitant for proteids, and hence the nicotin silicotungstate, when brought into the flask for steam distillation, may be largely contaminated with proteid matter. It is well known that proteid matter when distilled with steam at atmospheric pressure and in the presence of caustic alkalies may yield notable quantities of ammonia. The authors therefore employ calcined magnesia at this stage of their process.

It is true that magnesia has in the past received considerable recognition as the alkali to be employed when preexisting ammonia in organic matter is to be determined. In but comparatively few cases, however, has it yielded results above suspicion, and among chemists of the present day it appears quite generally recognized that proteid matter in general is subject to a certain amount of decomposition when distilled with magnesium oxid at atmospheric pressure, so that the substance is employed in such circumstances only provisionally for lack of a more convenient method, or when results of a comparative, rather than of an absolute, degree of accuracy are required.

On the whole, then, the method as employed by Bertrand and Javillier did not appear to embody everything desired by the bureau

¹ Bertrand, G., and Javillier, M. Sur le silicotungstate de nicotine et sur le dosage de cet alcaloïde. Bulletin des Sciences Pharmacologiques, tome 16, No. 1, pp. 7-14, Jan., 1909.

in a method worthy to replace the Kissling method, when applied to the examination of commercial nicotin preparations.

At the conclusion of their paper Bertrand and Javillier append the statement that in place of the volumetric method, which they prefer on account of its greater convenience, one may substitute a gravimetric method, in which the distilled nicotin is again precipitated with silicotungstic acid, the precipitate filtered, washed, and finally ignited. From the residue of SiO_2 and WO_3 may be calculated the weight of nicotin originally present. The authors quote no experimental work to show that this process is of more than theoretical applicability; nevertheless it appeared plausible and well worth investigating. Preliminary experiments afforded encouraging results, and a thorough study of the matter was undertaken.

Silicotungstic acid is a white to yellowish-white crystalline substance, presumably of the formula $4\text{H}_2\text{O} \cdot \text{SiO}_2 \cdot 12 \text{WO}_3 + 22\text{H}_2\text{O}$. When ignited it should leave, therefore, 85.87 per cent of a residue consisting of SiO_2 and WO_3 . Ignition of 1.5397 grams of the material employed in this laboratory gave a residue of 1.3981 grams, equal to 90.08 per cent. The acid itself, and its salts with the alkalis and ammonia, are readily soluble in acidulated water.

According to Bertrand and Javillier, nicotin silicotungstate dried at a temperature of 30°C . is a rose-white salt of the formula $2\text{C}_{10}\text{H}_{14}\text{N}_2 \cdot 2\text{H}_2\text{O} \cdot \text{SiO}_2 \cdot 12\text{WO}_3 + 5\text{H}_2\text{O}$, which loses its five molecules of water of crystallization when maintained for an hour at 120°C . The solubility of the hydrated salt in water has not been definitely determined, and, at any rate, it is very probably affected somewhat by the presence of acids and salts. The above authors, however, cite the limits at which a solution of the acid may serve as a qualitative test for nicotin. At a dilution of 1 in 300,000 of nicotin in the presence of one-tenth of 1 per cent concentrated hydrochloric acid, an almost immediate opalescence follows the addition of the reagent; less or more acid prevents an immediate visible reaction, though at 1 in 200,000 it appears in presence of four-tenths of 1 per cent of acid. These limits, however, are far from marking the true solubility of the salt, for at considerably greater dilutions and in presence of much more acid it will deposit on standing, not then as a cloud or an opalescence, but in definite crystalline form. The above-mentioned authors have obtained such crystals at a dilution of 1 in 1,000,000, but have not pushed their investigations farther.

Silicotungstic acid does not form such insoluble precipitates with all alkaloids, as indicated by recent work of Javillier.¹ Conin, for example, which is of interest in this connection for the reason that, like nicotin, it is volatile with steam, yields no precipitate with silicotungstic acid at dilutions greater than 1 in 5,000.

¹ Javillier, M. Sur les silicotungstates de conicine, de spartéine et d'atropine. Bulletin des Sciences Pharmacologiques, tome 17, No. 6, pp. 315-320, June, 1910.

In the case of some alkaloidal reagents, such as picric acid, a considerable quantity of the reagent must be present before any reaction is evident. Such appears not to be true of silicotungstic acid, for the present writer found that a 1 in 10,000 solution in one-tenth per cent hydrochloric acid yields an immediate opalescence upon the addition of one drop of a two-tenths per cent nicotine solution, increased to a heavy turbidity upon the addition of more nicotine. Hence nicotine is an appropriate reagent for the qualitative detection of small quantities of silicotungstic acid.

DEVELOPMENT OF THE QUANTITATIVE METHOD.

From the above observations it is evident that in quantitative work nicotine silicotungstate should be precipitated from a solution containing not less than one-tenth of 1 per cent of concentrated hydrochloric acid; that the precipitate should be allowed to stand before filtration until it has changed to a crystalline form; that the precipitate during filtration should be washed with water containing one-tenth of 1 per cent concentrated hydrochloric acid, and washing continued until the filtrate shows no opalescence when treated with a few drops of a dilute nicotine solution.

Since ammonia and amines are not precipitated by silicotungstic acid, the first step in a quantitative method for the determination of nicotine appeared to be the distillation of the solution or extract in a current of steam after addition of caustic soda. An aliquot of the distillate might then be precipitated with silicotungstic acid, and filtered and washed with the precautions noted above. The precipitate might then be ignited and the residue of SiO_2 and WO_3 weighed as suggested by Bertrand and Javillier, or the filtration might be performed in a Gooch crucible and the salt directly weighed after drying at 120°C . Both methods were tried.

Weighing as anhydrides.—The anhydrid method is obviously the quicker and easier of the two possibilities. A platinum crucible is naturally employed, from which the residue is easily removed. One drawback to the method results from the fact that when nicotine silicotungstate is decomposed by heat much carbon is deposited throughout the residual mass. If the ignition is performed carefully and with free access of air, such deposited carbon appears to be readily and completely burned away. The conditions are, however, evidently very favorable for the retention of unburned carbon or the reduction of WO_3 within the interior of the residue, which forms a coherent porous mass. Moreover, WO_3 is recognized to be appreciably volatile at a high temperature, hence protracted ignition of the residue must be avoided, though a high enough temperature must be reached to assure dehydration of silicic acid. Citation of an experiment performed will best serve to show the difficulties here involved. The precipitate obtained from about 0.06 gram of

nicotin was filtered on paper, washed, and the wet paper and precipitate brought into a platinum crucible. After drying and carbonization over a Bunsen burner the crucible was inclined and carbon burned away at a gradually increased heat. After cooling and weighing, the crucible and contents were next subjected to successive periods of heating, and were weighed after each period. The results are shown in Table 2.

TABLE 2.—*Effect of continued ignition upon the residue of anhydrids from nicotin silicotungstate.*

Method of ignition.	Weight of crucible and residue.
	<i>Grams.</i>
After ignition over Bunsen burner.....	20. 5029
After 5 additional minutes over Bunsen burner.....	20. 5024
After 5 additional minutes over Teclu burner.....	20. 5018
Do.....	20. 5015
Do.....	20. 5015
Do.....	20. 5012
After 20 to 25 additional minutes over Teclu burner.....	20. 5000
After 5 additional minutes over blast lamp.....	20. 4997

NOTE.—The crucible itself lost 0.0004 gram during the experiment.

It is evident that the weight of the residue changes appreciably on ignition, the degree and rate—and even the direction—of the change depending naturally on such circumstances as the conditions of heating and the degree of porosity of the residue. The results of Table 2 indicate that heating over a Teclu burner for a period between five and ten minutes will give the most consistent results in practice. The residue forms a porous mass, usually a clear lemon-yellow on the outside but showing a decided greenish hue in the interior.

Weighing as salt.—The salt method is subject to three disadvantages when compared to the anhydrid method: (1) A Gooch crucible must be prepared and weighed for each determination; (2) the precipitate packs down tightly upon the felt under suction, so that filtration is slow; (3) the anhydrous salt is very hygroscopic—a fact not mentioned by Bertrand and Javillier—hence the crucible and contents, after drying, must be tightly inclosed in a weighing bottle during cooling and weighing. On the other hand, results obtained by the salt method are free from the special sources of error attached to the anhydrid method. The salt reaches constant weight at 120° C. and may be held at 130° C. without showing further loss.¹ Hence the method is of value as an ultimate reference method in cases where very exact and indubitable results are necessary. For ordinary purposes the anhydrid method appears sufficiently accurate and is certainly much more rapid and convenient.

¹ The anhydrous salt theoretically contains 10.12 per cent nicotin. Before weighing the salt and Gooch crucible inclosed in the weighing bottle, the stopper of the latter must be loosened for an instant to permit equalization of air pressure.

ANALYTICAL RESULTS OBTAINED BY THE SILICOTUNGSTATE METHOD.

The silicotungstate method was first tested upon solutions of nicotin. The material bore the designation "highest purity" over the name of a firm of excellent reputation. That it was not absolutely pure was indicated by an evident opalescence when it was dissolved in dilute hydrochloric acid. A gram or two was weighed in a stoppered weighing bottle, washed into a measuring flask with slightly acidulated water, and made to volume. For the determination an aliquot was pipetted out, made to about 50 c. c., and sufficient dilute hydrochloric acid added to bring the amount present to between two-tenths and four-tenths of 1 per cent of concentrated hydrochloric acid. To the fluid so prepared was next added some excess of a silicotungstic acid solution, and the whole was well stirred and allowed to stand until the next day. By that time the precipitates, at first finely divided and settling very slowly, had become a mass of detached glittering crystals, settling quickly when agitated. The precipitate was filtered and washed with water containing 1 c. c. of concentrated hydrochloric acid in a liter, and then treated according to one or the other of the two methods outlined above. The results are given in the following table:

TABLE 3.—*Determination by silicotungstate method of nicotin in "Nicotin, highest purity."*

	Weight of nicotin taken.	Weight of anhydrous salt.	Weight of anhydrids.	Per cent nicotin.
	<i>Gram.</i>	<i>Gram.</i>	<i>Gram.</i>	
Solution A.....	0.05856	0.5103	99.34
	.058565119	99.65
	.05856	0.5761	99.56
	.05856	.5756	99.47
	.05856	.5777	99.83
	.11712	1.1505	99.42
Solution B.....	.05506	.5405	99.34

The results of the foregoing table do not justly show the precise limits within which duplicate determinations upon a single nicotin solution may be made to check, for in the work no special precautions were taken outside of ordinary laboratory practice. In taking aliquots of the nicotin solution the ordinary 25 c. c. pipettes supplied to the laboratory were employed indifferently, as they happened to come to hand on different days. These pipettes, though supplied by reputable makers, were not calibrated, and a subsequent test of some of the stock in hand showed that the variation in volumes delivered was nearly of as great magnitude as the variation in the percentages of nicotin found in the samples.

In the above series of determinations the volume of filtrate and washings was not definitely noted, though it evidently averaged about

150 c. c. To determine whether any restriction of or correction for the volume of filtrate and washings might be necessary, an aliquot of Solution A containing 0.05856 gram nicotin was precipitated and filtered as before upon an S. & S. No. 589 filter paper, 9 cm. in diameter, and washed in the ordinary manner until the total volume of filtered liquid amounted to 500 c. c. The weight of residue obtained after ignition was 0.5116 gram, equivalent to 99.59 per cent nicotin. A repetition of the experiment, continuing washing until the total volume amounted to 900 c. c., gave 0.5120 gram residue, equivalent to 99.67 per cent nicotin. It is hence apparent that the precipitate is extremely insoluble, that it may be precipitated quantitatively from very dilute solution, and that no correction for volume of filtrate or washings is necessary.

THE DETERMINATION OF NICOTIN IN COMMERCIAL PREPARATIONS.

If the silicotungstate method is destined to receive recognition and wide employment as a reliable and useful method for the determination of nicotin, a matter of considerable practical importance will be a determination of the degree of closeness with which results thereby obtained may be expected to check with those of the Kissling method when both methods are applied to the same commercial nicotin preparations. Several such preparations, which had previously been carefully and repeatedly analyzed by the Kissling method, were examined. The results are contained in the following table. It must be noted that each figure given in the last two columns of the table under the "silicotungstate method" represents a single complete independent analysis. The results in these two columns, then, are comparable as complete duplicates, and not merely as duplicate determinations upon a single nicotin distillate.

TABLE 4.—*Comparative results obtained by Kissling and silicotungstate methods when applied to commercial nicotin preparations.*

Character of preparation.	Per cent nicotin.		
	Kissling method (mean result).	Silicotungstate method.	
		Weighed as salt.	Weighed as anhydrids.
Nicotin dip, No. 660.....	21.86	21.91
Nicotin solution, No. 755.....	40.05	39.77
Tobacco extract, No. 768.....	8.36	8.32	39.84
Nicotin solution, No. 810.....	39.41	8.35
Tobacco extract, No. 842.....	2.94	40.12
			40.00
			2.92
			2.93

In view of the distinctly different rationale of the two methods and the difference in the sources of possible error inherent in each, the above series of results show a rather surprising agreement. Apparently the results obtained by one method are about equally likely to be either higher or lower than the results obtained by the other.

THE EFFECT OF EXTRANEOUS BASES.

As already noted, ammonia and amines—but not pyridin—may be practically eliminated by the Kissling method if a sufficiently long period of extraction is employed. Up to the present time apparently the only attention paid to the possible presence of pyridin bases in nicotin solutions is contained in the work of Emery,¹ performed in the laboratories of this bureau some years ago. Nicotin is levogyrotory, and Emery took advantage of this fact to work out a method for the polariscopic examination of nicotin distillates. Emery's method is not particularly accurate when considered as a method for the determination of nicotin, and the presence of pyridin bases can be inferred with assurance only if the amount of nicotin so found is notably lower than that obtained by the Kissling method.

Ammonia and amines, so far as known, are not precipitated by silicotungstic acid, nor are the anilins, as indicated by a test on a solution of anilin oil in dilute hydrochloric acid. Pure pyridin in slightly acid solution yielded a precipitate with silicotungstic acid at a dilution of 1 in 5,000 only when allowed to stand several hours; at a dilution of 1 in 10,000 no precipitate could be perceived after forty-eight hours. Pyridin bases from coal-tar creosote extracted in the laboratory proved to yield much less soluble precipitates, an immediate opalescence appearing upon the addition of silicotungstic acid to a solution of the bases containing, roughly, 1 part in 50,000, but no reaction could be observed at a dilution of 1 in 100,000. The precipitate formed from the higher pyridin bases was markedly different in appearance from nicotin silicotungstate, seeming to remain amorphous, and yielding a permanently cloudy fluid. It is therefore evident that the silicotungstate method can not be relied upon to eliminate more than small amounts of pyridin bases. It will, however, successfully detect such bases if present in quantity sufficient to introduce error in the determination of nicotin, for although the silicotungstates of some of the pyridin bases are difficultly soluble, they are much more soluble than nicotin silicotungstate; hence, if they are thrown on a filter and washed, the washings will long continue to yield a turbidity when tested with a dilute nicotin solution, exactly as would a very dilute solution of silicotungstic acid. To illustrate this point, 0.01 gram of the pyridin bases extracted from coal-tar creosote was completely precipitated from 50 c. c. of slightly

¹ Emery, James A. Estimation of nicotine in presence of pyridine. *Journal of the American Chemical Society*, vol. 26, No. 9, pp. 1113-1119, September, 1904.

acidulated solution with silicotungstic acid, and the precipitate brought on a filter and washed. When the volume of filtrate and washings amounted to over 500 c. c. fresh washings still yielded a faint but unmistakable opalescence when tested with a few drops of nicotin solution. The precipitate had then been reduced to a very small amount in the point of the filter and the experiment was discontinued.

Accordingly, it appears that the presence of pyridin bases in quantity sufficient to affect the accuracy of the determination of nicotin in commercial preparations will be betrayed by an apparent difficulty experienced in washing the precipitate free from excess of silicotungstic acid.

TESTS ON A RAPID MODIFICATION OF THE METHOD.

In ordinary work the natural system of routine will be to perform the steam distillation and precipitation on one day, leaving the precipitate to form and settle overnight. In exceptional cases, such as certain exigencies of factory control work, even so much delay may cause inconvenience. Hence an attempt was made to hasten the conversion of the precipitate into a filterable form.

A study was first made of the effect of various concentrations of hydrochloric acid and of various temperatures. The material employed was another sample of "pure nicotin" (sample C), evidently not so pure as those previously used. To about 1 gram of the material was added 5 c. c. of dilute hydrochloric acid (1 to 4) and the whole was made to 500 c. c. with distilled water. Portions of 50 c. c. were drawn with the same pipette for all determinations, and after dilution to 100 c. c. were acidified and precipitated with 10 c. c. of a 12 per cent solution of silicotungstic acid. The conditions of precipitation and the results obtained are given in the following table:

TABLE 5.—*Tests on rapid method of precipitation.*

No. of test.	Conditions of precipitation.	Weight of sample.	Weight of anhydrides.	Nicotin.
		<i>Gram.</i>	<i>Gram.</i>	<i>Per cent.</i>
1	Added 1 c. c. dilute HCl; stood 21 hours at 21° to 24° C. (recording thermometer); faintly cloudy when filtered.....	0.1034	0.8815	97.23
2	Added 2 c. c. dilute HCl; otherwise as above.....	.1034	.8824	97.33
3	Added 5 c. c. dilute HCl; otherwise as above.....	.1034	.8825	97.34
4	Added 10 c. c. dilute HCl; otherwise as above.....	.1034	.8834	97.44
5	Added 10 c. c. concentrated HCl; otherwise as above.....	.1034	.8802	97.09
6	Added 2 c. c. dilute HCl; stood about 18 hours at about 30° C....	.1047	.8943	97.34
7	Added 1 c. c. dilute HCl; stood 3 hours at 37.5° C., then in cold water for 15 minutes.....	.1034	.8809	97.17
8	Added 10 c. c. dilute HCl; otherwise as No. 7.....	.1034	.8809	97.17
9	Added 2 c. c. dilute HCl; on steam bath 20 minutes; in cold water 15 minutes.....	.1047	.8900	96.87
10	Added 2 c. c. dilute HCl; on steam bath 1 hour; in cold water 15 minutes.....	.1047	.8891	96.78
11	Added 1 c. c. dilute HCl; on steam bath 2 hours; in cold water 15 minutes.....	.1047	.8889	96.76
12	Added 2 c. c. dilute HCl; on steam bath $\frac{1}{2}$ hour; at room temperature 1 $\frac{1}{2}$ hours.....	.1034	.8776	96.80

Evidently precipitation may be successfully carried out in presence of considerable hydrochloric acid. In such case the liquid soon clears, but the crystals deposited are small and it is difficult to determine when the change to crystalline form is complete. Tests 2 and 3 made altogether much the best appearance and mark the limits of the amount of acid which should be added.

The obvious effect of heat in lowering the percentage of nicotin recovered was at first difficult to account for. Filtrates from such tests showed no further precipitation on long standing, and subsequent experiments in which the tests were allowed to stand over night before filtration showed similarly low results, namely, about 0.5 per cent lower than those obtained by precipitation at room temperature. The suspicion arose that the results obtained by precipitating hot were really the correct ones and that precipitates formed and settled at room temperature occluded silicotungstic acid. To settle this question portions of a nicotin solution were precipitated both hot and cold, the tests made cold of course being allowed to stand over night before filtration, while tests made hot were filtered after cooling for about one hour. The precipitates were filtered on paper, washed and dried. A portion of each precipitate was then placed in a weighed platinum crucible, dried to constant weight at 120° to 130° C., and the weight of the dried precipitate obtained. A weighing bottle was of course employed to hold crucible and precipitate during weighing. The crucibles and precipitates were next ignited, and the weights of the various residues were obtained. If the precipitate formed at room temperature occludes silicotungstic acid it should yield a residue greater than the theoretical and greater than the residue obtained from the precipitate formed in hot solution. That such was not the case is shown by the following results:

TABLE 6.—*Residues obtained by ignition of dried nicotin silicotungstate.*

Method of precipitation.	Weight of dried salt.	Weight of residue.	Residue on dried salt.
	<i>Grams.</i>	<i>Gram.</i>	<i>Per cent.</i>
Cold.....	1.008	0.8914	88.43
Do.....	.6902	.6105	88.45
Hot.....	.9848	.8724	88.59
Do.....	.6914	.6116	88.46

Theoretical per cent of residue=88.76.

The next set of experiments involved the formation and crystallization of the precipitate from a mechanically stirred liquid at room temperature, holding either nicotin or silicotungstic acid in excess throughout, so far as possible. Solution D of "pure nicotin" was employed. Results appear in Table 7.

TABLE 7.—*Results from precipitation of nicotin silicotungstate under various of "pure nicotin." Weight of sample taken for each test = 0.1019 gram.*

No. of test.	Conditions of precipitation.	Weight of anhydrids.		Nicotin.
		Gram.	Per cent.	
1	Volume of solution, 100 c. c.; added 2 c. c. dilute HCl; left at room temperature about 20 hours.	0.8662	96.91	
2	Volume of solution and acid, as in test 1; heated on steam bath $\frac{1}{2}$ hour, then left at room temperature overnight.	.8613	96.37	
3	Volume of solution and acid, as in tests 1 and 2; stirred mechanically overnight at room temperature.	.8657	96.85	
4	Volume of solution, 50 c. c.; added 1 c. c. dilute HCl; into this dropped from burette solution of 10 c. c. silicotungstic acid made to 50 c. c. and acidified with 1 c. c. dilute HCl, requiring 4 hours for complete addition of the reagent; liquid thoroughly stirred during addition of reagent and overnight; room temperature.	.8593	96.13	
5	Duplicate of test 4.	.8595	96.16	
6	Solution and reagent prepared as in tests 4 and 5, but precipitated under reverse conditions, i. e., by dropping the nicotin solution into the reagent; stirred overnight at room temperature.	.8650	96.77	

The results in Table 7 prove that the composition of the precipitate is variable. Either the precipitate formed in a cold solution in which silicotungstic acid is in excess contains too much silicotungstic acid or the precipitates formed in cold solutions in presence of excess of nicotin and in all hot solutions contain too much nicotin. The results shown in Table 6 render the first hypothesis unlikely. They do, however, permit the second hypothesis to be entertained, for if too much nicotin were in any case contained in the precipitate it is possible that excess nicotin might be volatilized during the process of drying at 120° to 130° C. and hence leave the dried precipitate of normal composition. Altogether the results given in Tables 6 and 7 indicate that quantitative results can be obtained only when nicotin is precipitated at not over 30° C. by an immediate excess of silicotungstic acid. The crystals of silicotungstate thus obtained appear to possess a composition which is uniform and practically that demanded by theory.

In performing the experiments noted in Table 7 it was observed that freshly precipitated nicotin silicotungstate very quickly became crystalline under the action of a mechanical stirrer. That thorough and continued agitation is all that is necessary to secure a simple and accurate rapid modification of the method is apparent from the following results:

TABLE 8.—*Tests on rapid method based on mechanical stirring, using solution D of "pure nicotin." Weight of sample taken for each test = 0.1019 gram.*

No. of test.	Conditions of precipitation.	Weight of anhydrids.		Nicotin.
		Gram.	Per cent.	
1	Volume of solution, 100 c. c.; added 2 c. c. dil. HCl and 10 c. c. silicotungstic acid; stirred over night. (Taken from Table 7, test 3, for comparison.)	0.8657	96.85	
2	As test 1, except that stirred $\frac{1}{2}$ hour only and filtered at once.	.8645	96.72	
3	As test 2, except that added 5 c. c. dil. HCl.	.8638	96.64	

In both test 2 and test 3 a change in the character of the precipitate had become apparent in the first 10 minutes; at the end of the second 10 minutes the change to a crystalline form appeared to be complete.

THE STEAM DISTILLATION OF NICOTIN PREPARATIONS.

Since nicotin is evolved from boiling dilute solutions with extreme slowness, in the steam distillation of nicotin preparations it is always desirable to keep the volume of liquid in the distillation flask at the lowest practicable amount. This is essential in the execution of the Kissling process, where the distillate is later to be titrated, and is there easily accomplished, since the liquid in the flask contains but a comparatively small amount of salts and other extraneous matter. Many commercial preparations contain a large amount of such extraneous matter, and during direct distillation consequently demand that a comparatively large volume of fluid be maintained in the distillation flask, with the result that a large volume of distillate must be collected in order to capture the last traces of nicotin. In general, this constitutes no serious drawback to the application of the silicotungstate method, for the extreme insolubility of nicotin silicotungstate admits of its quantitative separation from very dilute solutions of nicotin. The greater length of time required in such a direct steam distillation of a nicotin preparation is largely compensated in practice by the fact that when once properly started the distillation will progress without the close personal attention on the part of the analyst which is required during the steam distillation of the Kissling process, where the maintenance of the volume of liquid at its lowest possible amount is of great importance. In the case of high percentage nicotin solutions it is most convenient to count on a final volume of 1,000 c. c. for the distillate, in which case, if between 1 and 2 grams of nicotin are present in the whole, a 50 or 100 c. c. aliquot will be a proper and convenient amount to employ for precipitation. In the case of low percentage preparations the distillate may amount to 1,500 c. c. or more, and it is most conveniently handled by transferring to a 2,000 c. c. graduated stoppered cylinder and making to the next 100 c. c. mark.

In handling such low percentage preparations it is, of course, entirely possible, following the method of Bertrand and Javillier, first to precipitate the nicotin with silicotungstic acid, and then to subject the collected precipitate, after adding sufficient alkali, to distillation with steam, thus securing a small volume of liquid in the distillation flask and a consequent small volume of distillate. A serious practical objection to this method is the considerable necessary consumption of the expensive silicotungstic acid. It is possible that some other less costly material may be appropriate for such a preliminary precipitation, and in fact some preliminary experiments on the use of picric acid have been tried in this laboratory. But if it can possibly be avoided it seems very undesirable to introduce such a complication into a method which is otherwise consistent and uniform. At

any rate, the proper working out and testing of such a method would require considerable time. For the present, if it is necessary to handle any nicotin preparation which absolutely will not permit distillation with steam as the first step, it is always possible to extract the material with ether after the Kissling method—restricting the time of extraction to that necessary simply to extract all nicotin—and to proceed with the residue in the extraction flask according to the regular silicotungstate method.

SUMMARY AND CODIFIED DIRECTIONS FOR ROUTINE WORK.

It appears, then, that the silicotungstate method, a logical outgrowth from the work of Bertrand and Javillier, is placed upon a firm basis as a scientifically sound and technically practical method for the accurate and convenient determination of nicotin in nicotin solutions and tobacco extracts. The results obtainable by the method theoretically should be more accurate than the results afforded by the Kissling method, a hypothesis supported by the results of the experimental work herein cited. Each step in the process and the completeness of each operation may be tested as the work proceeds, and but little is left dependent upon the judgment of the operator. The method is likewise indicated to excel in convenience and economy, for though it employs an expensive reagent it demands but comparatively small quantities, and although it is a gravimetric method, it is simple and rapid, yielding definite results susceptible of close duplication with no excessive demands upon the time or skill of the analyst.

From the data of experimental work the following codified directions may be offered for the routine execution of analyses by the silicotungstate method:

Weigh out such an amount of the preparation as will contain preferably between 1 and 2 grams of nicotin, except in the case of extracts high in extraneous matter, where not in excess of 30 grams should be employed; wash with water into a 500 c. c. round-bottomed flask; add 1 to $1\frac{1}{2}$ grams paraffin, a few small pieces of pumice, and caustic soda solution to strong alkalinity (5 to 10 c. c. of NaOH solution, 1 to 2). Distill in a rapid current of steam through a three-bend connecting tube, a condenser, and adapter into 10 c. c. of dilute hydrochloric acid (1 to 4) in a capacious flask. When distillation is well started, apply heat to the distillation flask (a Babo funnel is most convenient) to reduce the volume of liquid as far as practicable without bumping or undue separation of insoluble matter. Continue distillation until a few cubic centimeters of the distillate collected from the condenser after removal of the adapter show no cloud or opalescence when treated with a drop of silicotungstic acid solution followed by a drop of dilute hydrochloric acid (1 to 4). Prove alkalinity of

the residue in the distillation flask with phenolphthalein solution or paper.

Make the distillate to convenient volume, mix well and pass through a large dry filter, discarding a liberal first portion of the filtrate, and test a portion with methyl orange to assure its acidity. Pipette into a beaker an aliquot containing about 0.1 gram nicotin, add for each 100 c. c. of liquid 3 c. c. of dilute hydrochloric acid (1 to 4)—or more, if indicated necessary by the test with methyl orange—and add 1 c. c. of a 12 per cent solution of silicotungstic acid for each 0.01 gram nicotin supposed to be present.¹ Stir thoroughly and let stand for eighteen hours.² Then stir up the precipitate, making sure that it settles quickly and completely in crystalline form; filter on a quantitative paper, and wash with cold water containing 1 c. c. of concentrated hydrochloric acid per liter. Test the first portion of the filtrate with a few drops of nicotin distillate to prove excess of silicotungstic acid.

Continue washing for two or three fillings of the filter after no more opalescence appears when a few cubic centimeters of fresh filtrate is tested with a few drops of nicotin distillate. Bring the wet paper and precipitate into a weighed platinum crucible, using a scrap of moistened filter paper to transfer any precipitate which may have crept up the sides of the funnel; dry carefully, carbonize, and finally burn off the carbon at as low a temperature as possible. Gradually increase the heat and occasionally rotate the crucible to expose all parts of the residue. At last ignite the inclined crucible thoroughly over the full heat of a Bunsen burner, finishing with five to ten minutes—not longer—over a powerful Teclu burner, or five minutes over a moderately powerful blast lamp. Cool in a desiccator. The weight of the residue multiplied by 0.114³ affords the weight of nicotin in the aliquot taken for precipitation. If the highest possible degree of accuracy is desired, filter the precipitate on a weighed Gooch crucible, dry at 125° C., and weigh the anhydrous nicotin silicotungstate as indicated on page 13.

¹ An excess of 17 to 18 per cent over the amount theoretically necessary is here provided, allowing for all ordinary variations.

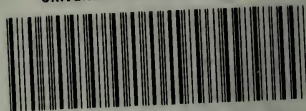
² This length of time is probably more than is necessary, but in the absence of positive evidence it is wiser to prescribe it. For quick results use a mechanical stirrer, as indicated on page 19.

³ The factor given by Bertrand and Javillier is 0.1139 and is apparently based upon an erroneous calculation, since other calculations cited by them check with those performed in this laboratory upon the basis of the most recent available atomic weights. The factor is actually slightly over 0.1140.





UNIVERSITY OF ILLINOIS-URBANA



3 0112 000596442